# Tools for Measuring the Performance of DNA Microarray Readers

*The NIST Gene Expression Metrology Team is developing a method and materials to establish DNA microarray scanner performance. Validation of signal measurement in microarray experiments provides quantitative, objective evidence that a scanner is performing consistently from day-to-day. More reliable DNA microarray scanners will enable better gene expression determinations to be performed and new innovations in medical diagnostics.*

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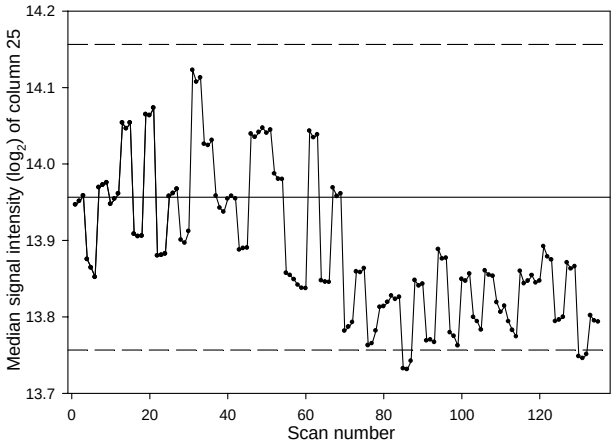
DNA microarrays have the potential to enable specific care based on an individual genome, bringing personalized medicine closer to reality. However, the measurement needs of the technology, including validation, uncertainty, and traceability, must be better understood and met before microarrays are accepted for use in clinical practice. One frequently overlooked source of variability in a microarray experiment is the microarray scanner.

Under ideal conditions the microarray scanner is expected to produce a stable response to a given material day after day, a response that matches that given on the previous day and with the previous scan, as well as those scans made in the future. However, without validation materials and a quantitative history of stable performance it is difficult to tell that a microarray scanner is not working correctly. Scanner validation would provide an on-going record of scanner performance allowing the user to determine whether the scanner is contributing an undue amount of uncertainty to a microarray experiment. At this time there are no well-characterized methods to track scanner performance or to establish comparability of different scanners.

## NIST quantitative DNA microarray scanner performance measures will help to establish the magnitude of variability in microarray experiments arising from scanner performance.

To validate microarray scanner performance a material that produces the same response under given conditions is required in order to judge the instrument performance independently from the performance of the material in use. Using dye slides with multiple identical rows of successive dilutions of Cy3 and Cy5 dyes, figures of merit including signal intensity, slope of the linear region, limit of detection, background, and signal-to-noise are under investigation. As an investigation of the suitability of this proposed tool for scanner validation, dye slides from three different lots (two "in-date," and "out-of-date", some slides stored desiccated per manufacturers specifications, and some stored in room air) have been scanned in triplicate daily, weekly, or at the beginning and end of the experimental period. The effects of a variety of factors on scanner performance over a five-week period as established with the dye slide were investigated. These factors (both slide- and scanner-related) include the effects of the number of scans, age of slide, slide lot, scanner unit, and scanner calibration on performance.




***Control chart of signal intensity of one concentration from the linear range of the Cy3 serial dilution series. Triplicate scans were made twice daily morning and afternoon over a five week period. The broken reference lines represent 3 standard deviations from the mean (solid line) of the first week's data.***

Tracking the figures of merit over time reveals whether they may be appropriate as measures of scanner performance. Significant differences in the Cy5 versus Cy3 dye performance are evident, with the Cy5 dye showing signs of degradation that make separation of the instrument performance from the dye performance difficult. The stability of the Cy3 slope and signal intensity of a column within the linear region observed with control charts indicates the potential of these figures of merit for scanner validation. An additional figure of merit, background, holds promise for use with both dyes, as a way of measuring scanner performance over time and determining if the associated instrument variability is acceptable. Through the use of these proposed figures of merit, a better understanding of the variability contributed by the microarray scanner to the overall experiment is possible.